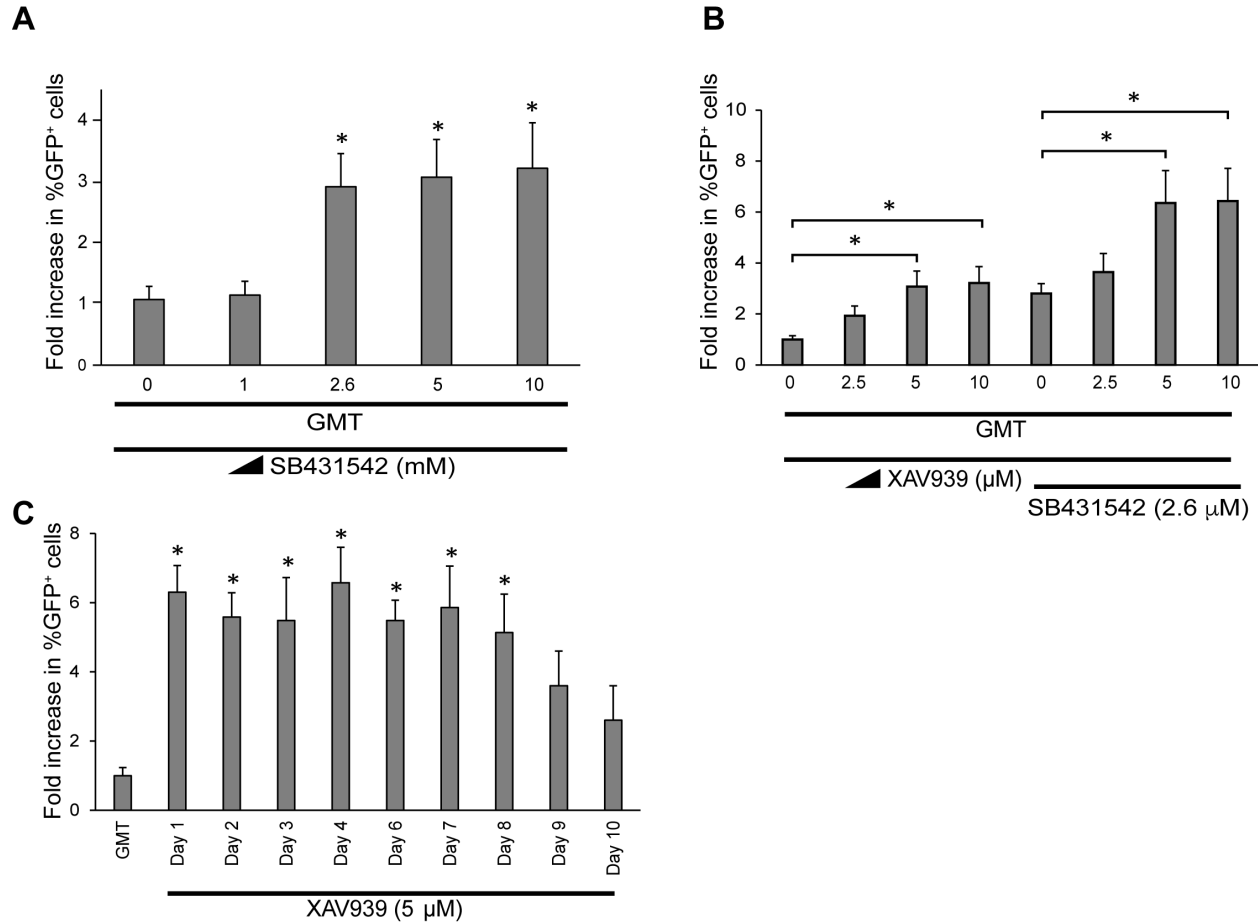
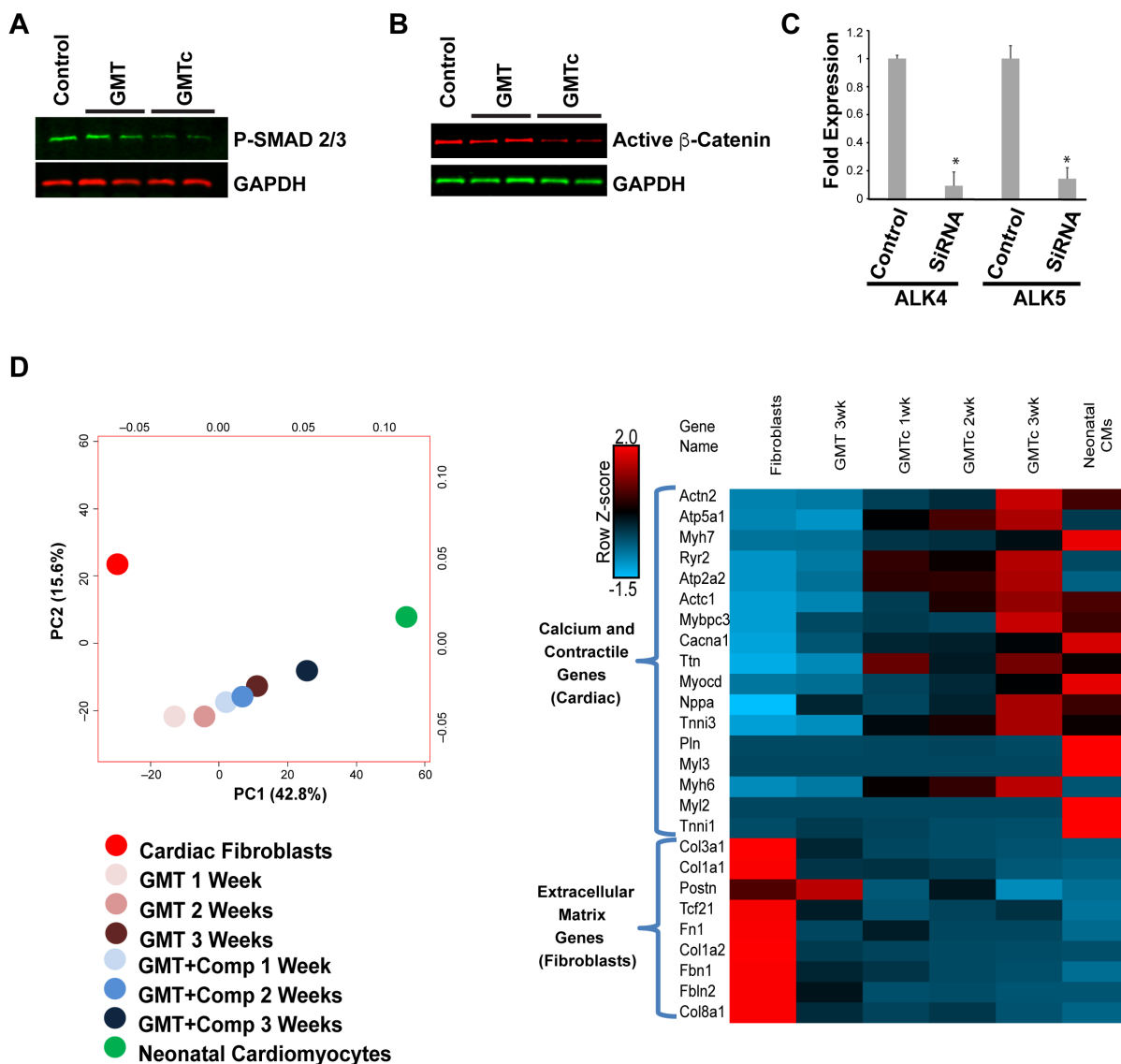


## SUPPLEMENTAL MATERIALS

### Supplementary Figures



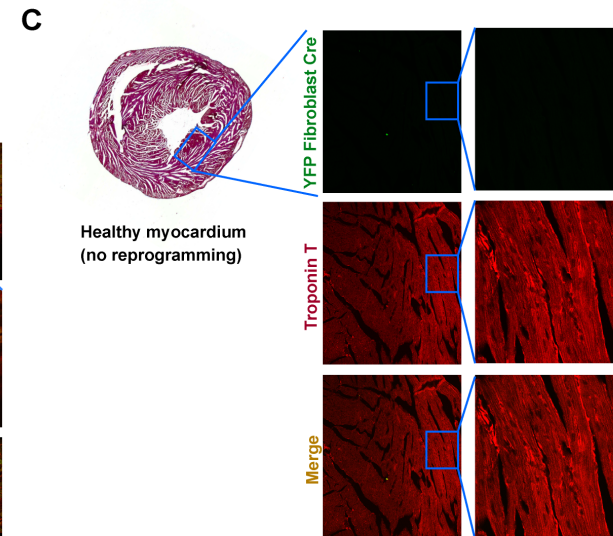
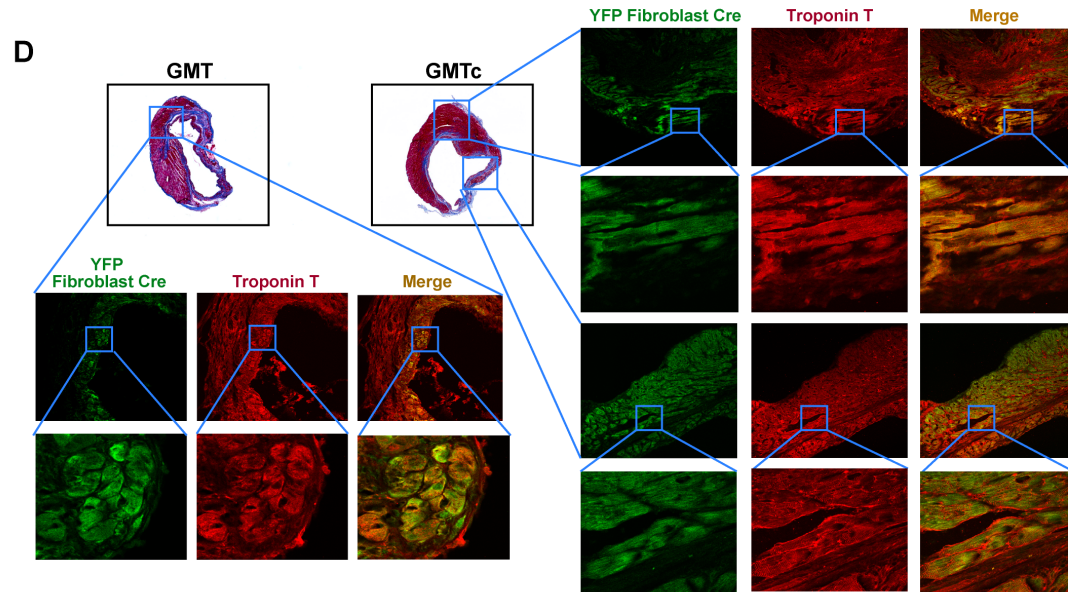
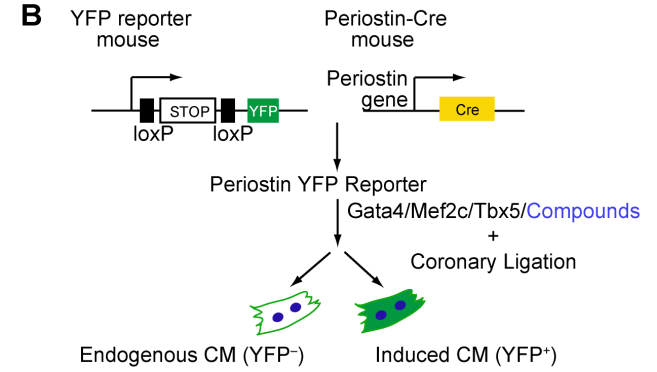
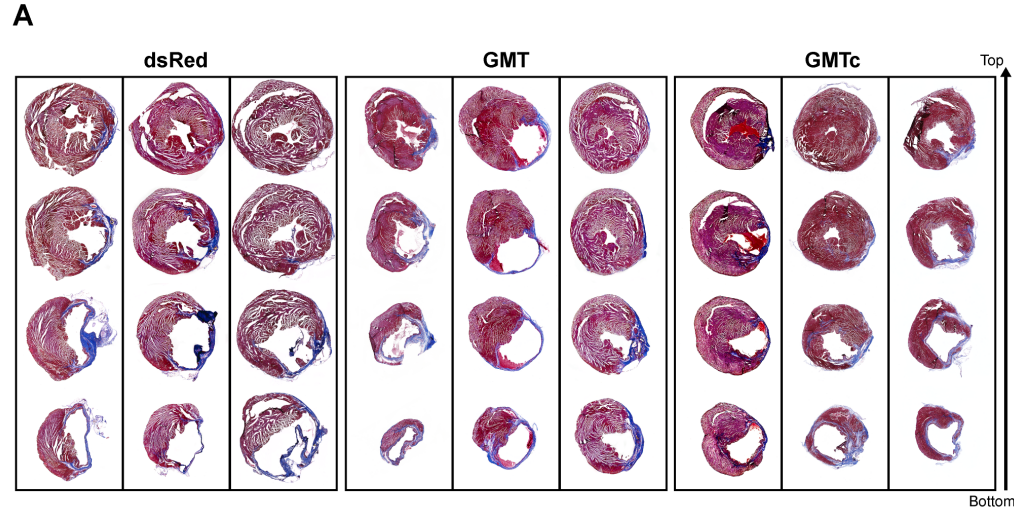
**Figure S1. Dosage and timing optimization for SB431542 and XAV939.** Bar graphs show the quantification of the effect of different dosages of SB431542 (**A**) and XAV939 on reprogramming efficiency ( $\alpha$ -MHC-GFP<sup>+</sup> cells) either in the presence or absence of SB431542 (**B**). (n=2 independent experiments with 3 technical replicates each, \*p<0.05). (**C**) Bar graph shows the effect of adding XAV939 at different time points after reprogramming (n=2 independent experiments with 3 technical replicates each, \*p<0.05).



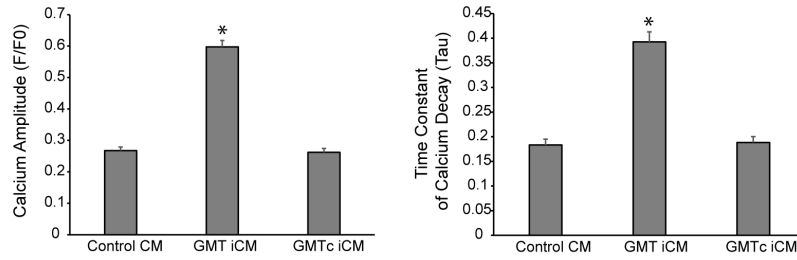
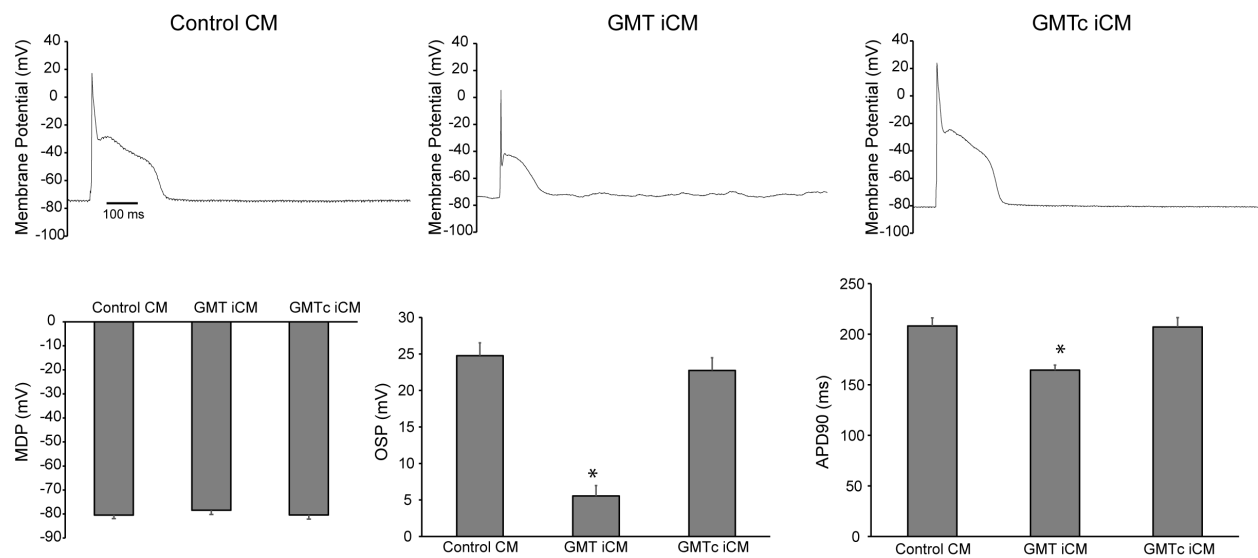
**Figure S2. SB431542 and XAV939 accelerate the reprogramming process.**

Representative western blot shows the (A) phosphorylation of SMAD2/3 as well as (B) the active form of  $\beta$ -Catenin expression were suppressed by the addition of the compounds (GMTc) compared to GMT. GAPDH levels served as loading controls. (C) qPCR shows efficiency of downregulation of ALK4 and ALK5 using siRNA (n=2, \*p<0.05). (D) Left panel shows Principal Component Analysis (PCA) plot for the

transcription profile assessed by RNAseq shows that GMT reprogrammed fibroblasts are at an intermediate state between fibroblasts neonatal cardiomyocytes and progress over time. However, iCMs reprogrammed with compounds have advanced further towards attaining a neonatal cardiomyocyte identity than time-mated iCMs receiving no compounds. Right panel shows a row normalized heat map of expression levels of selected cardiomyocyte and fibroblast genes that were not sufficiently regulated by GMT alone indicates advanced reprogramming progression over time in iCMs generated with compounds (GMTc).



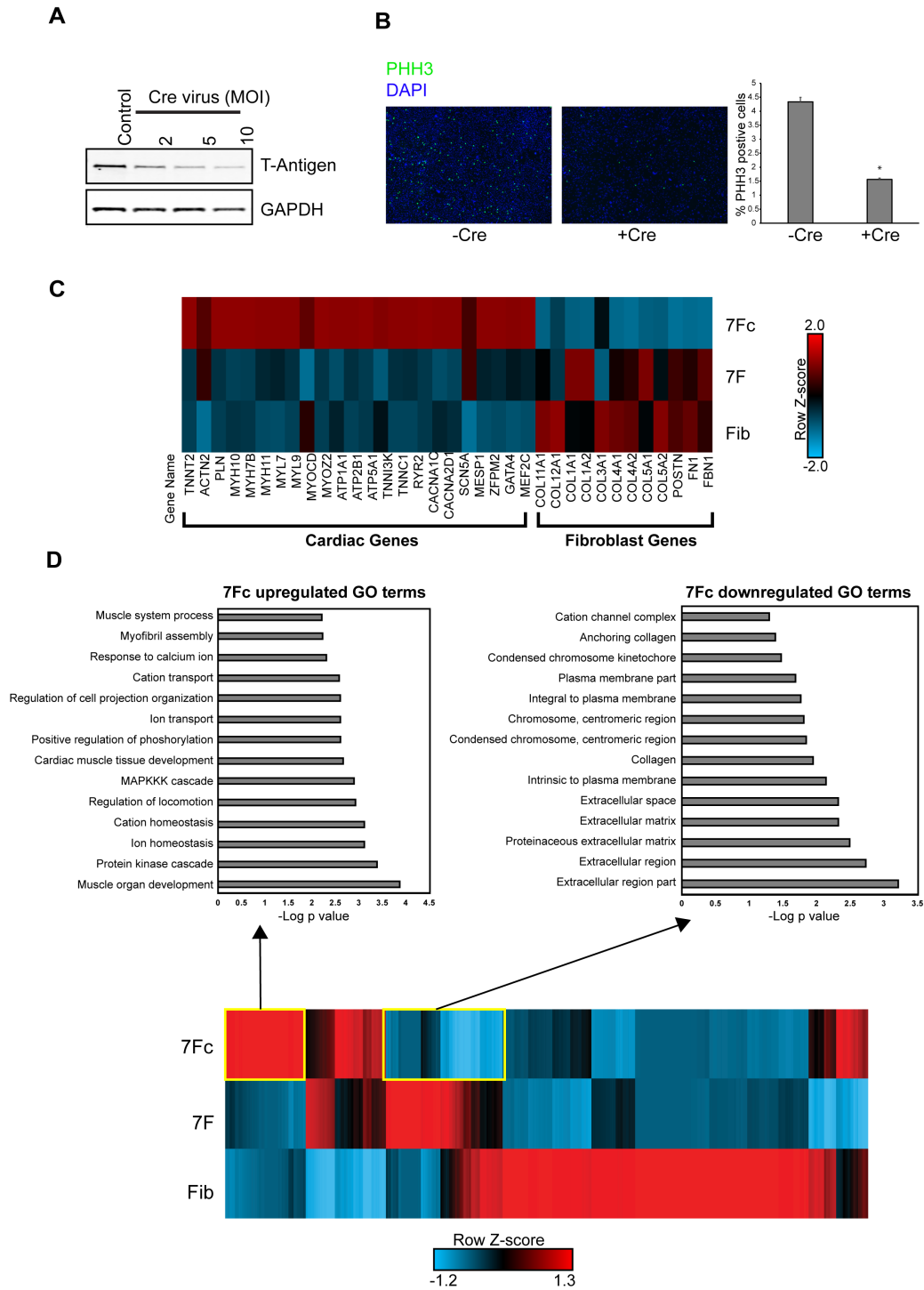
**Figure S3. *In vivo* reprogramming is more efficient in GMTc group compared to GMT.** (A) Representative range of histological sections with Masson's Trichrome staining for hearts after infarct treated with dsRed (control), GMT, or GMTc. Each vertical group represents a heart with sections from the apex (Bottom), most affected by infarct, to top part of the ventricle (Top). (B) Schematic of lineage tracing using ROSA-YFP/Periostin-Cre mice, to track the cell fate conversion of fibroblasts into cardiomyocytes. (C) Representative histological sections with Masson's Tri-Chrome staining for normal healthy myocardium away from the infarct site from GMTc treated heart. Corresponding immunofluorescence staining showing no detectable reprogrammed iCMs (double positive for Troponin-T (red) and the fibroblast cre-specific YFP reporter (green)). (D) Representative histological sections of apical sections with Masson's Trichrome staining or immunofluorescence staining showing that the remuscularization around the infarct area was due to newly formed iCMs in GMTc (positive for troponin T (red) and the fibroblast cre-specific YFP reporter (green)). Areas within infarct were largely YFP<sup>+</sup> and areas through the apical muscle were a mix of YFP<sup>+</sup> and YFP<sup>-</sup> cells.

**A****B**

**Figure S4. Quantification of calcium transients and electrophysiological parameters of in vivo iCMs**

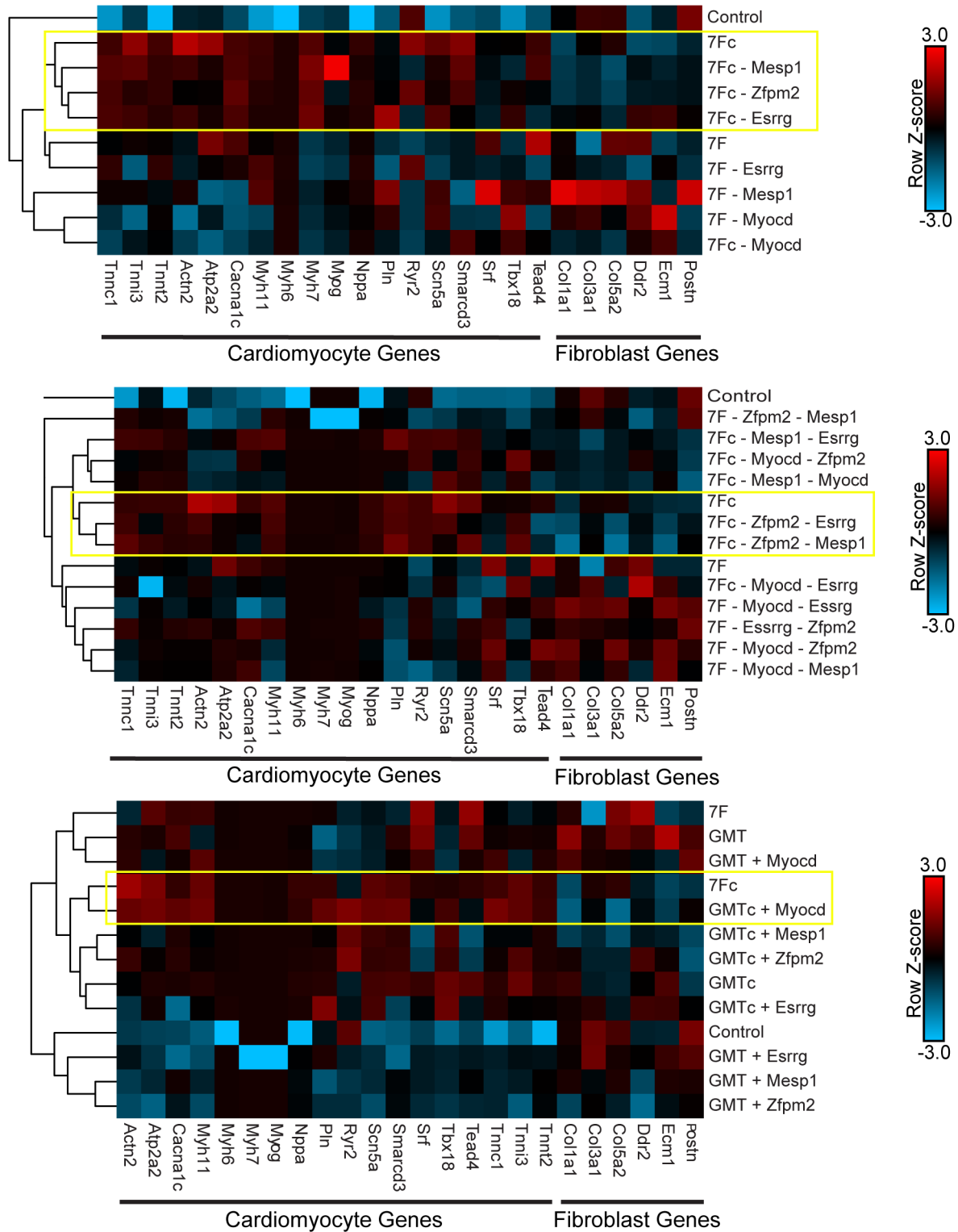
**(A)** Quantification of calcium amplitude and time constant of decay (Tau) in control cardiomyocytes (CM), GMT iCM and GMTc iCM (n=35 cells from 3 animals, \*p<0.05).

**(B)** Representative action potential recorded from control CM, GMT iCM and GMTc iCM and quantification of maximum diastolic potential (MDP), over shooting potential (OSP) and the time to reach 90% decline in action potential (APD90) (n=5, \*p<0.05).



**Figure S5. SB431542+XAV939 enhances human cardiac reprogramming using the 7 factors.** (A) Western Blot showing the downregulation of T-Antigen expression in human cardiac fibroblastss following infection with different MOIs of Cre virus. (B)

Representative images and quantification of human cardiac fibroblasts stained for the proliferation marker, phosphor histone H3 (PHH3) with and without Cre virus infection (n=3, p<0.05). **(C)** Row normalized Z-score heat map showing gene expression of major cardiac and fibroblast genes in 7F and 7Fc iCMs by RNA-seq. **(D)** Row normalized gene expression heat map and bar graphs for the top Gene Ontology term (GO) annotation for the differentially expressed genes between *in vivo* 7F iCMs and iCMs generated with 7F + SB431542 + XAV939 (7Fc).



**Figure S6. Replacement of the human cardiac reprogramming factors in the presence of SB431542+XAV939.** Row clustered heat maps showing gene expression

of a panel of cardiac and fibroblast genes by qRT-PCR when we removed one factor (upper panel), 2 factors (middle panel) or 3 factors (lower panel) from the 7 factor reprogramming cocktail in the presence or absence of SB431542 and XAV939. In the presence of SB431542 and XAV939, a similar degree of reprogramming occurred with four factors (Gata4, Mef2c, TBX5, and Myocardin (Myocd) (4Fc)) as did with seven factors (7Fc). Yellow boxes indicate conditions that clustered with 7Fc.

**Table S1: List of Taqman probes used in RT-PCR in human cardiac reprogramming**

Gene name	Taqman Probe Cat #
Gapdh	Hs99999905_m1
Kcna5	Hs00969279_s1
Gata4	Hs00171403_m1
Myocd	Hs00538071_m1
Myog	Hs01072232_m1
Myh7	Hs01110632_m1
Tead4	Hs01125032_m1
Mef2c	Hs00231149_m1
Tbx18	Hs01385457_m1
Smarcd3	Hs00162003_m1
Postn	Hs01566734_m1
Pln	Hs00160179_m1
Tnni3	Hs00165957_m1
Tnnc1	Hs00268524_m1
Srf	Hs00182371_m1
Nppa	Hs00383231_m1
Scn3a	Hs00165693_m1
Esrrg	Hs00155006_m1
Mesp1	Hs00251489_m1
Kcne1	Hs00897540_s1
Zfpm2	Hs00201397_m1
Col1a1	Hs00164004_m1
Ecm1	Hs00189435_m1
Ddr2	Hs01025953_m1
Cacna1c	Hs00167681_m1
Atp2a2	Hs00544877_m1
Myh11	Hs00224610_m1
Actn2	Hs00153809_m1
Tnnt2	Hs00943911_m1
Col3a1	Hs00943809_m1
Kcnd2	Hs01054873_m1
Col5a2	Hs00893878_m1
Ryr2	Hs00892883_m1
Tbx5	Hs00361155_m1
Rpl19	Hs02338565_gH

## **SUPPLEMENTARY MOVIES**

**Supplementary Movie 1.** GMTc 1 week beating iCMs

**Supplementary Movie 2.** GMTc 3 weeks beating iCMs

**Supplementary Movie 3.** GMTc iCM calcium transients in vitro

**Supplementary Movie 4.** Calcium transients from control adult cardiomyocytes

**Supplementary Movie 5.** Calcium transients from in vivo GMT iCM

**Supplementary Movie 6.** Calcium transients from in vivo GMTc iCM

**Supplementary Movie 7.** Calcium transients from human 7Fc iCM after 10 days

**Supplementary Movie 8.** Calcium transients from human 7Fc iCM after 3 weeks

**Supplementary Movie 9.** Calcium transients from human 4Fc iCM after 3 weeks

## SUPPLEMENTARY METHODS

### Drug Screening

Thy1+ neonatal cardiac fibroblasts from  $\alpha$ -MHC-GFP transgenic mice <sup>1</sup> were plated in 384-well plates at a density of 2000 cells/well. Cells were reprogrammed with the GMT retrovirus as described above. At day 1 of reprogramming, the virus was replaced with iCM medium and the compounds were added to the wells using a Biomek liquid-handling robotic station to achieve a 1:1000 dilution of the drug-library concentration. We screened libraries of 5500 toxicologically tested compounds (e.g., Ding Lab, LOPAC, TOCRIS, LONZA, and SPECTRUM). At day 14 of reprogramming, the plates were fixed and stained for GFP and Troponin T as described under immunocytochemistry and imaged using high-throughput, high-content imaging with an INCELL system. Data were analyzed using the INCELL image-analysis package.

Samples were fixed using 4% paraformaldehyde (PFA), washed twice in PBS containing 0.1% Triton X-100 (PBST), and blocked with Power Block Universal Blocking Reagent (Biogenex, #HK085-5K) for 15 minutes at room temperature as previously described <sup>2</sup>. Samples were incubated in a mixture containing primary antibodies diluted in PBST and blocking buffer (1:1) and incubated at 4°C overnight. The following primary antibodies were used: troponin T, cardiac isoform Ab-1, mouse monoclonal antibody (Thermo Fisher #MS-295-PO, 1:200), anti-PHH3 (Abcam AB5176, 1:200) and anti-GFP (Thermo Fisher #A11120, 1:200). Samples were washed twice with PBST for 15 minutes each at room temperature and then incubated in a mixture containing secondary antibodies diluted in PBST and blocking buffer (1:1) at room temperature for 1 hour. Samples were washed three times with PBST for 15 minutes each at room temperature

and visualized. Stained cells were quantified with ImageJ Imaging Analysis Software. The standard error mean was calculated for all comparisons;  $p < 0.05$  was considered statistically significant.

### **FACS Analyses and Sorting**

For GFP expression analysis, reprogrammed cardiac fibroblasts were harvested from cultured dishes and analyzed on the LSR-II (BD) with FlowJo software. For live-cell sorting and analysis, cells were dissociated using TRYPLE (Thermo Fisher) and sorted using an LSRII (BD) machine. For fixed-cell FACS, cells were fixed with formalin for 15 min, permeabilized with 0.1% v/v Triton X-100, and stained with anti-GFP (Thermo Fisher) and either anti-Troponin-T (Thermo Fisher) antibodies, followed by secondary antibodies conjugated with Alexa-594 (Thermo Fisher).

For live-cell sorting for RNAseq,  $\alpha$ -MHC-GFP mouse cardiac fibroblasts or hTNT-GFP<sup>+</sup> reprogrammed human cardiac fibroblasts were dissociated from cultured dishes and sorted for GFP<sup>+</sup> expression with an Aria II FACS sorter.

### **Western Blotting**

Proteins from cell lysates containing 20  $\mu$ g of protein were separated by SDS-PAGE and electrophoretically transferred onto polyvinylidene difluoride membranes (BioRad). Membranes were then washed in PBS, treated with blocking buffer (Li-cor), and then incubated with primary antibodies at 4°C overnight. The following primary antibodies were used: anti-GAPDH antibody loading control (Abcam #Ab9484, 1:1000), anti-Active  $\beta$ -Catenin antibody (Millipore #05-665, 1:1000), anti-T-Antigen (Abcam #ab16879, 1:1000),

and anti-phospho-Smad2/3 (Cell Signaling #8685S, 1:1000). Membranes were washed in PBS and incubated with the appropriate secondary antibodies (Li-Cor, IRDye 600LT; IRDye 800CW, 1:10,000) for 1 hour at room temperature. Membranes were washed and visualized with an Odyssey Fc Dual-mode Imaging System

### **Real-time PCR**

Total RNA was isolated with a commercial kit (Direct-Zol RNA Mini-prep, Zymo Research). Reverse transcription was carried out using a mixture of oligo(dT) and random hexamer primers (SuperScript III First-Strand Synthesis SuperMix for qRT-PCR, ThermoFisher Scientific). Real-time PCR Analysis was conducted with the 7900HT FAST real-time PCR detection system (Applied Biosystems). Taqman probes used are listed in **Table S1**. For high-throughput, real-time PCR analysis, samples were analyzed with the 96.96 Dynamic Array IFC on the Biomark HD system (Fluidigm).

### **RNAseq Analyses**

$\alpha$ -MHC-GFP<sup>+</sup> in vitro mouse iCMs, TNT-GFP<sup>+</sup> human iCMs, and control dsRed infected cardiac fibroblasts (mock) were sorted with a FACS Aria II cell sorter. After Langendorff isolation of the in vivo reprogrammed iCMs, the iCMs were picked based on their expression of Periostin cre YFP reporter using micropipette. Their RNA was isolated using the Qiagen, miRNeasy Micro Kit #210874. Using the Ovation RNA-seq System v2 Kit (NuGEN), the total RNA (20–50 ng) was reverse transcribed to synthesize first-strand cDNA using a combination of random hexamers and a poly-T chimeric primer. The RNA template was then partially degraded by heating and the second-strand cDNA was

synthesized using DNA polymerase. Double-stranded DNA was then amplified using single primer isothermal amplification (SPIA). SPIA is a linear cDNA amplification process in which RNase H degrades RNA in DNA/RNA heteroduplex at the 5'-end of the double-stranded DNA, after which the SPIA primer binds to the cDNA, and the polymerase starts replication at the 3'-end of the primer by displacing the existing forward strand. Random hexamers were then used to linearly amplify the second-strand cDNA. cDNA samples were fragmented to an average size of 200 bp using the Covaris S2 sonicator. Libraries were made from the fragmented cDNA using the Ovation Ultralow V2 kit (NuGen). Following end repair and ligation, the libraries were PCR amplified with 9 cycles. Library quality was assessed by a Bioanalyzer on High-Sensitivity DNA chips (Agilent) and concentration was quantified by qPCR (KAPA)<sup>3, 4</sup>. The libraries were sequenced on a HiSeq 2500 sequencer with a single-read, 50-cycle sequencing run (Illumina). We utilized the RNAseq-analysis pipeline reported previously<sup>5</sup>. For the readers' convenience and completeness of the current manuscript, we review the important steps/features and/or statistics of the pipeline below. Known adapters and low-quality regions of reads were trimmed using Fastq-mcf (<http://code.google.com/p/ea-utils>). Sample QC was assessed using FastQC (<http://www.bioinformatics.babraham.ac.uk/projects/fastqc/>). Reads were aligned to the mouse-reference assembly *mm9* or human-reference assembly *hg19* using Tophat 2.0.13<sup>6</sup>. Gene expression was tallied by Subread featureCounts<sup>7</sup> using Ensembl's gene annotation for *mm9* or *hg19*. Finally, we calculated differential expression P-values using edgeR<sup>8</sup>. Here, we first filtered out any genes without at least two samples with a CPM (counts per million) between 0.5 and 5000. CPMs below 0.5 indicates non-detectable gene expression, and CPM above 5000 is typically only seen in mitochondrial

genes. If these high-expression genes were not excluded, their counts would disproportionately affect the normalization. After excluding these genes, we re-normalized the remaining ones using “calcNormFactors” in *edgeR*, then calculated P-values for each gene with differential expression between samples using *edgeR*’s assumed negative–binomial distribution of gene expression. We calculated the false discovery rates (FDRs) for each P-value with the Benjamini-Hochberg method <sup>9</sup> based on the built-in R function “p.adjust”. We used AltAnalyze version 2.0.9 for generating the heatmaps of the relative gene expression of WNT and TGF $\beta$  signaling genes <sup>10</sup>. All default options in this software were used.

### **Animal experiments**

Postn-Cre:R26R-YFP mice were obtained by crossing Postn-Cre mice and Rosa26-EYFP mice. The animal protocol for surgery was approved by the University of California, San Francisco Institutional Animal Care and Use Committee. All surgeries were performed as previously described <sup>11</sup>. Briefly, 8-9 week old mice were anaesthetized with 2.4% isoflurane/97.6% oxygen and placed in a supine position on a heating pad (37°C). Animals were intubated with a 19 G stump needle and ventilated with room air using a MiniVent Type 845 mouse ventilator (Hugo Sachs Elektronik-Harvard Apparatus; stroke volume, 250  $\mu$ l; respiratory rate, 120 breaths per minute). Myocardial infarction (MI) was induced by permanent ligation of the left anterior descending (LAD) artery with a 7-0 prolene suture as described <sup>11</sup>. Sham-operated animals served as surgical controls and were subjected to the same procedures as the experimental animals with the exception that the LAD was not ligated. The animals were randomized into 4 different groups (8

mice in each group) GMT, GMT + Compounds, DsRed, and DsRed + Compounds. A pool of concentrated virus (GMT) was mixed, and 10 µl of mixed virus and 10 µl of PBS was injected into the myocardium through an insulin syringe with an incorporated 29-G needle (BD). Injection with a full dosage was carried out along the boundary between the infarct zone and border zone based on the blanched infarct area after coronary artery occlusion. At days 2 through 15, the animals received daily intraperitoneal injections of SB431542 (10 mg/kg/day) and XAV939 (2.5 mg/kg/day).

Serial echocardiography was conducted before MI and 1, 2, 4, 8 and 12 weeks after MI to assess the cardiac function. Echocardiography was performed blindly with the Vevo 770 High-Resolution Micro-Imaging System (VisualSonics) with a 15-MHz linear-array ultrasound transducer. The left ventricle was assessed in both parasternal long-axis and short-axis views at a frame rate of 120 Hz. End-systole or end-diastole were defined as the phases in which the left ventricle appeared the smallest and largest, respectively, and used for ejection-fraction measurements. To calculate the shortening fraction, left-ventricular end-systolic and end-diastolic diameters were measured from the left ventricular M-mode tracing with a sweep speed of 50 mm/s at the papillary muscle. B-mode was used for two-dimensional measurements of end-systolic and end-diastolic dimensions.

At the end of the experiments (12 weeks after MI), animals were exposed to magnetic resonance imaging (MRI) to assess cardiac structure and function. This assessment was followed by sacrificing the animals and harvesting the hearts for histological studies. Standard Masson's Trichrome staining was performed on hearts 12 weeks post-viral delivery and coronary artery ligation. To determine scar size, we used ImagePro software

to measure the scar area (blue) and healthy area (red) on transverse sections spanning four levels within the left ventricle of an MI heart. From each level, we measured four slices of tissues as technical quadruplicates (for a total of 16 sections).

## **MRI**

At the end of 12 weeks, *in vivo* MRI imaging was performed blindly with a 7T preclinical horizontal bore magnet interfaced with an Agilent imaging console. Animals were anaesthetized by inhalation of 2% isoflurane with 98% oxygen and placed into a home-built, linear-polarized birdcage coil with 28-mm internal diameter (ID). Body temperatures during imaging experiments were kept at 34°C. Heartbeats, breathing, and temperature of the animals were monitored with an MRI-compatible, small-animal life-support system (SA Instruments Inc, Stony Brook, NY). Location and long and short axes of mouse hearts were determined from scout images with the following parameters: repetition time (TR), 10 msec; echo time (TE), 4 msec; excitation flip angle, 20-degree; field of view (FOV), 6 cm<sup>2</sup>; matrix dimension, 128x128; slice thickness, 1 mm; number of repetitions, 2. To evaluate functional parameters of control and infarcted hearts, MRI images of short axes were acquired with a spin echo–pulse sequence. Parameters of the acquisition were TR=1 sec, TE=10 msec, in-plane image resolution=200 µm, and acquisition time=~20 min (depended on heart rate). Cardiac and breathing gating were employed. To measure the ejection fraction, nine or ten short-axis slices of the heart were acquired at diastole (zero delay after R-heart-peak) and systole (45% of the R-R interval delay from the R-peak).

## Isolation of adult CMs

Adult cardiomyocytes (CMs) were isolated as described <sup>11</sup>, with minor modifications. Briefly, adult mice were anaesthetized with isoflurane and mechanically ventilated. Hearts were removed and perfused retrogradely via aortic cannulation with a constant flow of 3 ml/min in a Langendorf apparatus. Hearts were perfused at 37°C for 5 min with Wittenberg Isolation Medium (WIM) containing 116 mM NaCl, 5.4 mM KCl, 6.7 mM MgCl<sub>2</sub>, 12 mM glucose, 2 mM glutamine, 3.5 mM NaHCO<sub>3</sub>, 1.5 mM KH<sub>2</sub>PO<sub>4</sub>, 1.0 mM NaH<sub>2</sub>PO<sub>4</sub>, 21 HEPES, with 1.5 nM insulin, essential vitamins (GIBCO), and essential mM amino acids (GIBCO) (pH 7.4), followed by digestion solution (WIM supplemented with 0.8 mg/ml collagenase II and 10 µM CaCl<sub>2</sub>) for 10 min. Hearts were then removed from the Langendorf apparatus while intact (with tissues loosely connected). Desired areas (i.e., border/infarct zone) were then micro-dissected under the microscope, mechanically dissociated, triturated, and resuspended in a low-calcium solution (WIM supplemented with 5 mg/ml bovine serum albumin, 10 mM taurine, and 150 µM CaCl<sub>2</sub>). Cells were then spun at low speed, supernatant was removed, and calcium was gradually reintroduced through a series of washes. For calcium transient measurements, cells were used on the same day as isolation and, until recordings, were stored at room temperature (21°C) in M199 (Gibco) supplemented with 5 mM creatine, 2 mM L-carnitine, 5 mM taurine, and 1.5 nM insulin. For immunohistochemistry, cells were plated onto laminin-coated culture slides, allowed to adhere, and fixed on the day of isolation. For RNAseq, contractility analysis, and calcium-transients measurements, iCMs were selected manually by micro-pipette based on the presence

of Periostin-Cre:R26R-YFP signal under the fluorescent microscope immediately after isolation.

### **Calcium-transient assessment**

Calcium transients were assessed as previously described <sup>11</sup>. Briefly, isolated myocytes were loaded with Fluo-4 for 30 min at room temperature before being transferred to the superfusion chamber. The loading solution contained a 1:10 mixture of 5 mM Fluo-4 AM in dry DMSO and Powerload<sup>TM</sup> concentrate (Invitrogen), which was diluted 100-fold into extracellular Tyrode's solution containing suspended myocytes. An additional 20 min was allowed for de-esterification before recordings were taken. Contractions and calcium transients were evoked by applying voltage pulses at 1 Hz between platinum wires placed on either side of the cell of interest and connected to a field stimulator (IonOptix, Myopacer). Fluo-4 fluorescence transients were recorded via a standard filter set (#49011 ET, Chroma Technology). Upon binding calcium, the fluorescence intensity of Fluo-4 increases >100-fold which allowed recording over the basal GFP or YFP fluorescence in the iCMs. Resting fluorescence was recorded after cessation of pacing, and background light was obtained after picking up and removing the cell from the field of view at the end of the experiment.

### **Action potential recordings**

Action potential recordings were performed as described before in <sup>11</sup>. Briefly, Isolated myocytes suspended in tissue culture medium were transferred to a superfusion chamber (RC-26GLP; Warner Instruments) on the stage of a Nikon TiS inverted

fluorescence microscope equipped with a dual wavelength microfluorometer (IonOptix). Myocytes isolated from either periostin-Cre:R26R-YFP hearts were identified as control CMs or iCMs on the basis of YFP fluorescence. The myocytes were whole cell patch-clamped using an Axopatch 200B amplifier and pClamp software (Molecular Devices). Patch electrodes of 2–5 MV (1B-150F; WPI) were filled with intracellular solution containing 120mM KCl, 20mM NaHEPES, 10mM MgATP, 5mMK2EGTA (or 0.1 mM), 2mMMgCl<sub>2</sub>, and adjusted to pH 7.1 with KOH. The cells were superfused with a modified Tyrode's extracellular solution containing 137mMNaCl, 10mMNaHEPES, 10mMdextrose, 5mMKCl, 2mM CaCl<sub>2</sub>, 1mM MgCl<sub>2</sub>, adjusted to pH 7.4 with NaOH. After GV seal formation, whole-cell access to the myocyte was established by applying brief pressure pulses, and the amplifier was switched to current clamp mode, whereupon the cell's resting potential developed. Action potentials were stimulated at 0.33 Hz using 2 nA, 2 ms current pulses applied through the patch pipette, and were signal averaged in tens. All membrane potentials were corrected for a 25.6mV liquid junction potential determined via pClamp software. Finally, the amplifier was switched back to voltage clamp mode to identify individual ion channel currents as required. In some experiments, the concentration of K2EGTA in the intracellular solution was reduced to 0.1mM to permit excitation–contraction coupling to occur, and 100 mM K5Fluo-4 (Invitrogen Corp) was added to define the presence of cytosolic calcium transients during action potentials. Electrophysiology data were digitized at 5 kHz and low-pass filtered at 2 kHz. Analysis was performed using pClamp, Microsoft Excel, and Origin (OriginLab) software. Action potential duration was measured from the point of

maximum depolarizing voltage change (DV/Dt<sub>max</sub>) to 50, 70 and 90% repolarization. Unless stated, experiments were performed at room temperature.

### **Human Cardiac Reprogramming**

Ventricular human cardiac fibroblasts (HCF) were purchased from Lonza. To generate the stable immortalized cell line, we infected the cells with floxed human T-antigen lentivirus, which contains a puromycin-selection cassette (Addgene plasmid #18922). Cells were selected using 1 µg/ml puromycin for 5 days. Cells were then infected with Cre virus before reprogramming to cut out the T-antigen. Reprogramming was conducted as previously described <sup>12</sup>. Briefly, pMXs retroviral vectors encoding the seven human cardiac developmental factors (Gata4, Mef2c, Tbx5, Myocardin, Esrrg, Mesp1, Znfpm2) were transfected into Platinum-A (Cell Biolabs) cells to generate viruses. After 48 h, HCFs were transduced overnight with the pool of virus containing supernatants and supplemented with 6 µg/ml polybrene. Media was then replaced with iCM media [DMEM:M199 (4:1), 10% FBS, 1x non-essential amino acids (NEAA), 1x penicillin/streptomycin] containing SB431542 (2.6 µM) at 24 h post-infection and XAV939 (5 µM) 48 h post-infection. For visualization of reprogramming and cell sorting, reprogrammed HCFs were either transduced with plx-hTNT-GFP or plx-hTNT-GCaMP5. Briefly, plx-hTNT-GFP or plx-hTNT-GCaMP5 were transfected into HEK 293FT cells with Eugene HD along with the lentivirus packaging plasmids pMD2.G and psPAX2 to generate the lentivirus. After 48 h, HCFs were transduced overnight and supplemented with 6 µg/ml polybrene. At day 4 of reprogramming, RPMI1640 with B27 supplement was

added every 3 days at 25% increments with iCMs until it replaced iCM media completely at day 15.

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